

# REPORT DOCUMENTATION PAGE

Form Approved  
OBM No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE June 1999		3. REPORT TYPE AND DATES COVERED Proceedings	
4. TITLE AND SUBTITLE Spatial and Temporal Relationships Between Localized Corrosion and Bacterial Activity on Iron-Containing Substrata				5. FUNDING NUMBERS Job Order No. Program Element No. 0601153N Project No. 03103 Task No. 320 Accession No. DN094463	
6. AUTHOR(S) M. Franklin*, B.J. Little and R.I. Ray					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Naval Research Laboratory Oceanography Division Stennis Space Center, MS 39529-5004				8. PERFORMING ORGANIZATION REPORT NUMBER NRL/PP/7303--99-0032	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research 800 N. Quincy Street Arlington, VA 22217-5000				10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
19990830 088					
11. SUPPLEMENTARY NOTES Fourth International European Federation of Corrosion Workshop on Microbial Corrosion, 6-9 June 1999, Lisboa, Portugal *Microbiology Department, Montana State University, Bozeman, MT 59717					
12a. DISTRIBUTION/AVAILABILITY STATEMENT  Approved for public release; distribution is unlimited.				12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words)  A series of laboratory and field experiments were designed to determine the temporal and spatial relationships between accumulations of bacteria and pitting corrosion of iron-containing metals exposed in fresh and marine electrolytes. Abiotic corrosion was established in both carbon and stainless steels prior to the introduction of viable and glutaraldehyde-fixed bacteria in fresh water and seawater media. In all cases a spatial relationship between accumulations of cells and localized corrosion was documented, regardless of the origin of the localized corrosion. Both viable and glutaraldehyde-fixed cells were preferentially attracted to anodic regions on iron substrata and cells were enmeshed in iron corrosion products. The attraction, specific for iron, was more influential than topography in determining the spatial distribution of bacterial cells. Results indicate that spatial relationships cannot be interpreted as causal, i.e., accumulations of bacterial cells in iron corrosion products cannot be simply interpreted as microbiologically influenced corrosion.					
14. SUBJECT TERMS MIC, spatial relationships, temporal relationships, localized corrosion, abiotic corrosion, iron corrosion, pit initiation, OCP (open-circuit potential), metabolic activity, aerobic heterotrophic bacteria, carbon steel, and glutaraldehyde-fixed cells				15. NUMBER OF PAGES 4	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT SAR		

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

DTIC QUALITY INSPECTED 4



INSTITUTO  
SUPERIOR  
TÉCNICO

# **FOURTH INTERNATIONAL EUROPEAN FEDERATION OF CORROSION WORKSHOP ON MICROBIAL CORROSION**

*[234th Event of the European Federation of Corrosion]*

Lisboa, Portugal  
June 6-9, 1999

## **Workshop Organisers:**

C.A.C. Sequeira  
Instituto Superior Técnico  
Portugal

A.K. Tiller  
Corrosion Centre  
U.K.

D. Thierry  
Swedish Corrosion Institute  
Sweden

## **ABSTRACTS BOOK**

Reproduced From  
Best Available Copy

# Spatial and Temporal Relationships Between Localized Corrosion and Bacterial Activity on Iron-Containing Substrata

M. Franklin,<sup>1</sup> B. J. Little<sup>2†</sup> and R. I. Ray<sup>2</sup>

1) Microbiology Department, Montana State University, Bozeman MT 59717

2) Naval Research Laboratory, Stennis Space Center, MS 39529

† Corresponding Author

## ABSTRACT

A series of laboratory and field experiments were designed to determine the temporal and spatial relationships between accumulations of bacteria and pitting corrosion of iron-containing metals exposed in fresh and marine electrolytes. Abiotic corrosion was established in both carbon and stainless steels prior to the introduction of viable and glutaraldehyde-fixed bacteria in fresh water and seawater media. In all cases a spatial relationship between accumulations of cells and localized corrosion was documented, regardless of the origin of the localized corrosion. Both viable and glutaraldehyde-fixed cells were preferentially attracted to anodic regions on iron substrata and cells were enmeshed in iron corrosion products. The attraction, specific for iron, was more influential than topography in determining the spatial distribution of bacterial cells. Results indicate that spatial relationships cannot be interpreted as causal, i.e., accumulations of bacterial cells in iron corrosion products cannot be simply interpreted as microbiologically influenced corrosion.

Experiments with *Pseudomonas* sp. were used to explore the significance of bacterial cells to sustained pit formation on carbon steel. *Pseudomonas* sp. does not grow anaerobically, and does not produce detectable levels of volatile fatty acids when grown on glucose. The bacterium produces copious amounts of extracellular polysaccharide when attached to a steel surface, as demonstrated by Fourier transform infrared spectroscopy and electron microscopy. Pit initiation and passivation were followed using open-circuit potential (OCP) measurements and current density mapping by scanning vibrating electrode (SVE). SVE techniques provide a non-destructive means to define the magnitude and sign of current densities in solution over freely corroding metals. Bacterial biosynthetic activity was resolved by incubating bacteria with <sup>14</sup>C labeled metabolic precursors followed by exposure to X-ray film.

Conditions in the sterile medium favored repassivation (Figure 1). The medium contained chloride, sulfate and phosphate ions at concentrations of approximately 1, 0.2 and 0.2 mM, respectively. Air was continuously bubbled through the media. An independent investigation determined that phosphate with aeration or stirring produced repassivation and inhibited propagating pits. In contrast, the presence or the metabolic activity, of aerobic heterotrophic bacteria had a marked effect on the corrosion of the carbon steel in a system containing the same

medium. The OCP, rather than remaining above -200 mV(SCE) as in the sterile control, slowly dropped to a value of less than -550 mV(SCE) in the presence of viable cells. The drop in OCP was not likely due to depletion of oxygen by bacterial respiration, since the medium was continually bubbled with air. Bacterial growth was also limited by the supply of glucose, 0.28 mM. These conditions should not lead an anaerobic environment.

Biosynthetic activity indicated by developed silver grains of autoradiograms corresponded most strongly with tubercles formed on the carbon steel, and with anodic activity observed by the SVE. Results of continuous flow experiments with *Pseudomonas* sp. exposed to carbon steel, clearly demonstrated that metabolically active bacteria were associated with anodic sites. The data cannot be unambiguously interpreted as to whether bacteria initiated the anodic site or were attracted to corrosion products. However in experiments in which bacteria were labeled prior to exposure to established anodic areas metabolically active cells were preferentially bound to abiotically-generated corrosion products.

Pit propagation, observed with either viable or glutaraldehyde-fixed cells, requires maintenance of a critical level of aggressive ions inside a pit. Dissolution current acts to increase the concentration of ions inside pits, and hydrolysis of ferrous ions maintains a low pH. In competition, diffusion of aggressive ions from pits reduces their concentration. Bacterial colonization may produce membranes that inhibit diffusion of aggressive ions from pits and/or diffusion of passivating ions, such as phosphate into pits. Propagation of pits was more rapid when viable bacteria were present than in the presence of glutaraldehyde-fixed bacteria. Rapid pit propagation in the presence of the viable bacteria may be due to the synthesis of cellular material and exopolymers over active anodic sites.

The role of microorganisms in causing pitting corrosion has traditionally been defined as one of initiation, i.e., the presence and activities of the organisms initiate an oxygen concentration cell and pit propagation is then controlled by hydrolysis reactions controlled by the composition of the metal. These experiments indicate that the sequence of events is subtly different and that the role of the microorganisms is in fact related to propagation. In fact, bacteria may be required for localized corrosion in some media.

Spatial and temporal relationships between *Pseudomonas* sp. and localized corrosion of carbon steel in a phosphate-containing medium can be summarized as follows: (1) Anodic sites form and repassivate in the absence of bacterial cells (2) Bacterial cells are attracted to anodic regions formed simultaneously with their growth or to established anodic sites. (3) Once bacteria become associated an anodic region repassivation is unlikely. (4) The presence and activities of microorganisms fix anodic sites that produce localized corrosion. (5) The time required for pit propagation depends on bacterial viability.

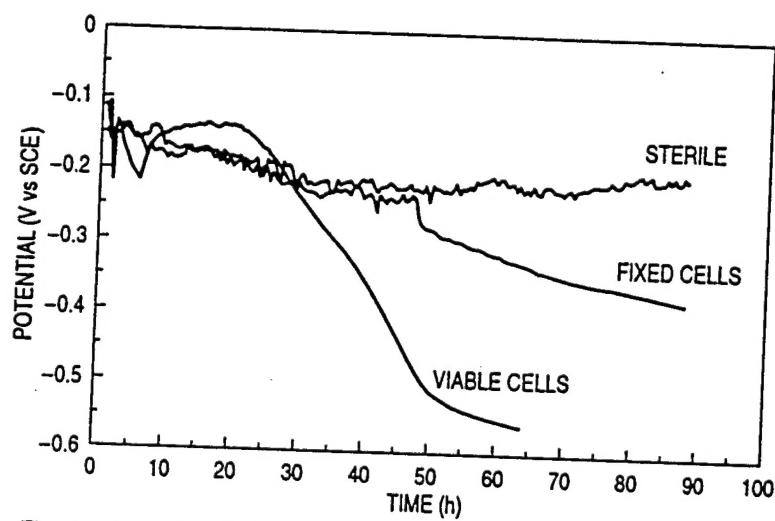


Fig. 1 - Potential field maps over carbon steel sample exposed to biofilm. Anodic sites initiated and repassivated until one site failed to repassivate and continued to propagate. The potential dropped as pit propagation occurred.